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PRINCIPAL INVESTIGATOR: Yunbo Liu
Pei Zhong

CONTRACTING ORGANIZATION: Duke University
Durham NC 27708

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Yunbo Liu
Pei Zhong

Email: yunboliu@hotmail.com

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14. ABSTRACT

This investigation aims to test the hypothesis that HIFU not only can destroy primary tumor tissues by thermal ablation but also is capable of inducing therapeutic effects in sub-lethally injured tumor cells via the heat shock response. During the current funding period, we have fully developed an image-guided, computer-controlled experimental HIFU system and characterized itscorresponding acoustic and thermal fields. The efficiency of HIFU-induced maker gene (GFP) activation under the control of hsp70B promoter was investigated in vitro by using a mice breast cancer cell line (4T1). Furthermore, a 3D cell-embedded tissue mimicking phantom was developed, which possesses similar acoustic and thermal properties to that of breast tissues. Using this 3D phantom model, we have performed preliminary experiments to determine the correlation between the spatial thermal dose distribution and resultant gene activation during HIFU treatment. Future work will focus on completion of the HIFU-induced gene activation in the 3-D tissue mimicking phantom and on gene activation during HIFU thermal ablation in vivo.

15. SUBJECT TERMS
HIFU, thermal ablation, heat shock response, gene activation

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Table of Contents

INTRODUCTION:

In recent years, high-intensity focused ultrasound (HIFU) has emerged as a new and promising non-invasive treatment modality for breast cancers. The fundamental principle of HIFU is to convert focused acoustic energy into heat and thus produce well-defined focal lesions inside the target tissue via thermal ablation (temperature > 65°C)[1]. Despite its great potential, a critical limitation of the current HIFU therapy is that it cannot eradicate 100% of the tumor volume due to inhomogeneities in tissue property and heat conduction. Furthermore, HIFU cannot be used to treat metastatic cancer cells outside the primary tumor site, which is a major cause of mortality following HIFU therapy.[2] The overall goal of this investigation is therefore to explore the potential to combine synergistically HIFU thermal ablation with heat-induced gene therapy (via the control of hsp70B-heat shock promoter) to improve the overall efficiency of breast cancer treatment. In the first year of the project, we proposed to fully develop and characterize an image-guided, computer-controlled experimental HIFU system; test the feasibility and efficiency of HIFU-induced green fluorescence protein (GFP) gene activation in 4T1 breast cancers *in vitro* in cell suspension; and to develop a 3-D cell-embedded tissue mimicking phantom to investigate the correlation between *in situ* thermal dose and gene activation during HIFU treatment.

BODY:

Image-guided experimental HIFU system: An experimental HIFU system was designed and constructed by integrating a single element air-back annular HIFU transducer (H-102, Sonic Concepts, fundamental frequency: 1.1-MHz, third harmonic: 3.3-MHz) with a portable ultrasound imaging system (Terason2000 with a 5/7 MHz phased array probe). The HIFU transducer was mounted either on the side-wall (for *in vitro* experiments) or at the bottom (for *in vivo* experiments) of a Lucite water tank (Fig.1a). A specially designed adapter was used to align the ultrasound imaging probe perpendicular to the HIFU beam at 0^o or 90^o orientation. The pressure output (Fig. 1b) at the focal point of the HIFU transducer was determined by using a fiber optic probe hydrophone (FOPH-500, RP Acoustics). A 0.1 mm bare ware thermocouple (IT-23, Physitemp Inc, Clifton, NJ) was used to measure the temperature increase produced by a 5s HIFU exposure *in vitro* (Fig. 1c).

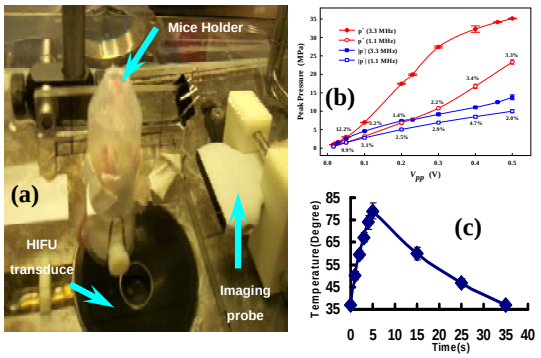

Fig. 1. Experimental HIFU system (a), pressure distribution (b), and temperature profile (c).

HIFU-induced gene (GFP) activation in cell suspension: The goal of this experiment was to determine the thermal dose for *in vitro* HIFU induced trans-gene expression in 4T1 cells (mouse mammary cancer cell), in preparation for HIFU-induced gene activation in animal models to be carried out in year 2. One day before HIFU treatment, 4T1 cells were transfected with Adeno-hsp70B-GFP adenovirus vector at a MOI (Multiplicity Of Infection = ratio of infectious virus particles to cells) of 10. For the HIFU exposure, a volume of 10-μl cell suspension ($5x10^7$ cells/ml) was loaded in a 0.2 ml Polymerase Chain Reaction (PCR) thin-wall tube, which was placed vertically with its conical bottom aligned within the –6 dB HIFU beam focus.[3]

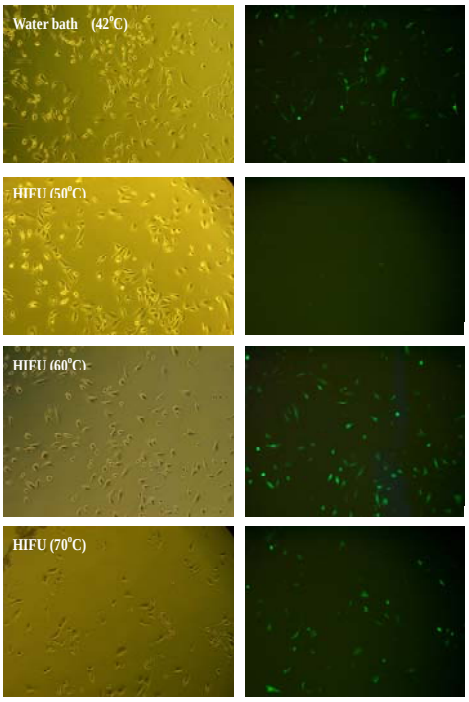

Fig. 2. Representative phase contrast (left column) and fluorescence (right column) images of 4T1 cells after 5s HIFU exposure at different peak temperatures. The images in the top are results from hyperthermia treatment (30 min).

The effects of peak temperature (50~70°C) on gene activation in the transfected 4T1 cells for a fixed heat shock exposure time of 5s were first evaluated by phase contrast and fluorescence microscopy (Fig. 2). A low level of GFP expression (4%) was induced at 50°C peak temperature and its GFP intensity increased about 3-fold compared to the control group (Fig. 3a). As the peak temperature increased to 60°C and 70°C, much stronger GFP expression (38% and 41%) was induced with concomitantly enhanced expression intensity of 35-fold and 42-fold, respectively. Furthermore, the effect of treatment duration on HIFU-induced gene activation at 60°C peak temperature shows a saturation after 5s exposure (Fig. 3b).

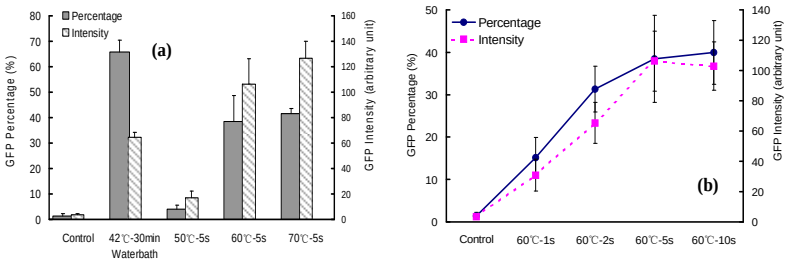

Figure 3. Gene activation at different peak temperatures (a) and different exposure durations (b).

HIFU-induced gene (GFP) activation in cell-embedded tissue phantom: To better understand the mechanism of HIFU-induced gene activation, it is desirable to develop a transparent 3D tissue-mimicking phantom with uniformly dispersed cancer cells so that the spatial temperature distribution, HIFU lesion formation, and gene expression can be assessed and correlated. We constructed a phantom matrix using 2% low gelling point (LGP) agarose (A9045, Sigma-Aldrich, St. Louis, MO). Thermally sensitive Bovine Serum Albumin (BSA) proteins (A7096, Sigma-Aldrich, St. Louis, MO) were incorporated (3~9%) into the agarose matrix in order to enhance its acoustic absorption and to facilitate lesion visualization. Glycerol (G7757, Sigma Co., St. Louis, MO) was also added (5%) to match the sound speed of the phantom with that of mammalian tissues. Finally, rat mammary carcinoma cells (R3230Ac), stably transfected with GFP plasmid under the control of hsp70B promoter, were mixed in the gel phantom solution at a concentration of 10^6/ml. The physical properties (acoustic, thermal and mechanical) of the phantom were characterized at room temperature based upon established methods and summarized in Table 1. Most of the physical properties of the tissue mimicking phantom are close to soft tissues except that the attenuation coefficient and nonlinear B/A parameter are comparably lower. Besides the compressive modulus and attenuation coefficient, sound speed, B/A ratio and thermal properties are independent of the BSA concentration (3~9%) and all the physical parameters examined are independent of cell concentration from 0.5 to 5 x 10^6/ml (data not shown).

Table 1. Physical properties of cell-embedded ultrasound phantom (20°C)

Physical properties	Attenuation Coefficient α (dB $cm^{-1}MHz^{-1}$)	Sound Speed C (m s^{-1})	Nonlinear Parameter B/A	Elastic Modulus E (KPa)	Thermal Conductivity κ (W m^{-1} C^{-1})	Thermal Diffusivity D (mm^2 s^{-1})
Soft tissues	0.5	1540	6.8 - 8.5	10 - 120	0.47 - 0. 6	0.12 - 0.15
US phantom	0.17	1500	5.5	4.7 – 8.7	0.56	0.13

Gene activation in the cell-embedded phantom was examined following HIFU exposure at I_{SATA} = 3570 W/cm^2 for 10s. A white thermal lesion spot was produced in the focal plane (Fig. 4a), which remained detectable (Dia = 0.5 mm) by phase contrast microscopy in 24 hours (Fig. 4b). In the boundary area surrounding the lesion (Fig. 4c), GFP-positive cells could be detected by fluorescence microscopy (Fig. 4d). Specifically, gene activation area was found primarily at 1.5 mm away from the lesion boundary. Using a series of fluorescent images obtained, gene activation pattern within the entire focal plane was reconstructed and shown in Fig. 5a. GFP-positive cells were observed mainly within a circular band (gene activation ring) located concentrically along the necrosis lesion boundary with an inner and outer diameter of about 1.5 and 2.0 mm from lesion center, respectively. Outside this specific area, gene expression could be barely detected in the cross section. Quantitatively, the average-peak temperature reached 97°C at the focal point and decreased progressively along the radial direction to ambient temperature (37°C) at 4 mm (Fig. 5b). The corresponding peak temperature in the gene activation zone was found to vary from 63°C at 1.5 mm radius to 54°C at 2 mm radius with a medium peak temperature of 58°C.

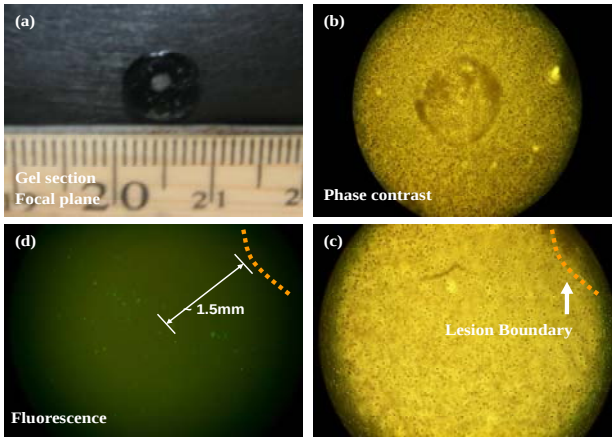

Fig. 4. (a) Gel section across the focal plane, (b) corresponding phase contrast image of HIFU lesion, (c) thermal lesion boundary, and (d) GFP gene activation distribution revealed by fluorescence microscopy.

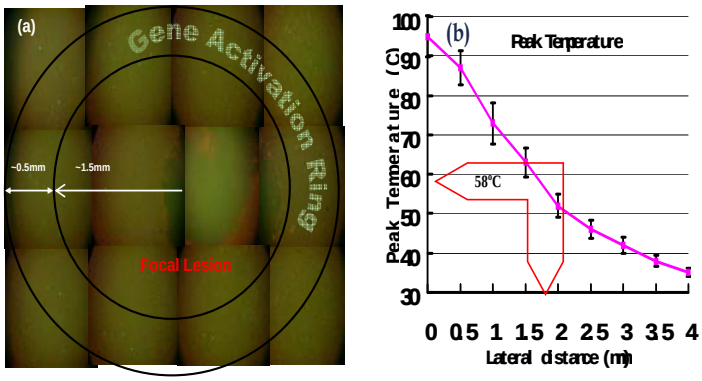

Fig. 5. (a) Gene activation ring within the focal plane and (b) peak temperature vs. lateral distance. Peak temperature varies between 63°C and 54°C (medium 58°C) in the gene activation ring.

KEY RESEARCH ACCOMPLISHMENTS:

- Integrated and characterized an image-guided experimental HIFU exposure system for *in vitro* and *in vivo* studies.
- Investigated the dose dependency of HIFU-induced gene (GFP) activation in 4T1 cell suspension.
- Developed and characterized a 3D cell-embedded transparent tissue mimicking phantom.
- Correlated gene activation with spatial temperature distribution across HIFU focal plane using the 3D cell-embedded tissue phantom.

REPORTABLE OUTCOMES:

1. **Liu Y**, Zhong P. High intensity focused ultrasound induced trans-gene activation in a cell-embedded tissue mimicking phantom. *IEEE Ultrasonics Symposium*, Vancouver, Canada, 2006

2. **Liu Y**, Zhong P. Development of a cell-embedded tissue mimicking ultrasound phantom. *Acoustical Society of America 151st meeting*, Providence, RI, 2006

CONCLUSION:

In the current funding period (4/06-3/07), an image-guided, computer-controlled experimental HIFU system was fully developed and characterized for both *in vivo* and *in vitro* studies. The efficiency of HIFU -induced gene (GFP) activation under the control of hsp70B promoter was investigated using 4T1 cells in suspension *in vitro*. To elucidate the underlying physical mechanism, a 3D cell-embedded tissue mimicking phantom was developed, which possesses similar acoustic and thermal properties with breast tissue. Using this 3D phantom model, the spatial temperature distribution and *in situ* gene activation could be correlated. Based on these encouraging preliminary results, our future work will focus on completion of the spatial correlation between HIFU-induced thermal dose and gene activation in the tissue mimicking phantom and combination of HIFU thermal ablation with heat-regulated IL-12 gene therapy to be tested in a 4T1 murine tumor model.[4]

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